



**UNIVERSITI PUTRA MALAYSIA**

**BIOASSAY-GUIDED ISOLATION AND IDENTIFICATION OF  
BIOACTIVE COMPOUNDS FROM GARCINIA PENANGIANA LEAVES**

**MOHD LIP BIN JABIT.**

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**By**

**MOHD LIP BIN JABIT**

**Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia,  
in Fulfilment of the Requirement for the Degree of Master of Science**

**December 2005**



## **DEDICATION**

My family

&

Friends

Many thanks for your support and inspiration

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

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**Chairman : Professor Nordin Hj Lajis, PhD**

**Institute : Bioscience**

Preliminary screening was done on 18 extracts of different parts of *Garcinia sp.* These extracts were tested on cytotoxic assay by using MTT Tetrazolium method on MCF-7 cells (hormone dependent breast cancer cells), DU145 (prostate cancer cells), H460 (non-small lung cancer) and HL60 (Leukemic cancer cells). Extracts of *G. penangiana* leaves, *Garcinia urophylla* leaves, *G. maingayi* leaves, *G. maingayi* stems and *G. opaca* fruits were found to have potent cytotoxic activity on MCF-7 cells and their IC<sub>50</sub> values are 5, 3, 6, 10 and 8 µg/mL, respectively. Furthermore, the extract of *G. penangiana* leaves also showed potent cytotoxic activity towards H460 cells (IC<sub>50</sub> value of 8 µg/mL).

Bioassay guided isolation and purification led to the isolation of five xanthenes and triterpene compounds from *Garcinia penangiana* leaves extracts. The triterpene and sterol isolated were characterized as friedelin (23) and stigmasterol (24), respectively. The two isolated xanthenes from the hexane fraction were characterized as 1,3,5,8-tetrahydroxy-4-(1,1-dimethyl allyl)xanthone (25) and cudraticusxanthone H (26). The

two xanthenes isolated from the dichloromethane extract were characterised as 1,3,5,6-tetrahydroxy-2-(1,1-dimethylallyl)-4-(3-methyl-2-butenyl)xanthone or macluraxanthone C (**27**) and the new penangianaxanthone (**25**). Compound designated as **29** was also found as a mixture of **27** in dichloromethane fraction. The biosynthesis of **25**, **26** and **28** was suggested in the discussion. These compounds were tested for cytotoxic assay by using MTT Tetrazolium method on MCF-7 cells (hormone dependent breast cancer cells), DU145 (prostate cancer cells) and H460 (non-small lung cancer cells). Compound **25**, **26**, **27**, **28** and mixture of **29** and **27** exhibited good and potent cytotoxic activity on MCF-7, NCI-H460 and DU145 cell lines. However, **23** and **24** showed no activity toward MCF-7 and NCI-H460 cell lines. **26**, **27**, **28** and mixture of **29** and **27** showed similar pattern of cytotoxic activity toward MCF-7 cell line with  $IC_{50}$  values of  $3.9 \pm 0.8$ ,  $3.1 \pm 0.1$ ,  $5.8 \pm 1.2$  and  $3.0 \pm 0.2$   $\mu\text{g/mL}$ , respectively. Similar patterns of cytotoxic activity were also observed when **25**, **26**, **27**, **28** and mixture of **29** and **27** tested on NCI-H460 cell lines. The compounds showed  $IC_{50}$  values of  $13.4 \pm 1.1$   $\mu\text{g/mL}$ ,  $5.0 \pm 1.2$   $\mu\text{g/mL}$ ,  $1.4 \pm 0.9$   $\mu\text{g/mL}$ ,  $4.5 \pm 1.4$  and  $2.0 \pm 0.7$   $\mu\text{g/mL}$ , respectively. **26**, **27**, **28** and mixture of **29** and **27** showed similar pattern of cytotoxic activity toward DU145 cell line with their  $IC_{50}$  values of  $4.6 \pm 0.2$ ,  $2.6 \pm 0.6$ ,  $4.3 \pm 0.4$   $\mu\text{g/mL}$  and  $3.0 \pm 0.4$   $\mu\text{g/mL}$ , respectively.



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Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

**PENGASINGAN BERPANDUKAN BIOCERAKIN DAN PENGENALPASTIAN  
SEBATIAN-SEBATIAN BIOAKTIF DARIPADA DAUN *GARCINIA*  
*PENANGIANA*.**

Oleh

**MOHD LIP BIN JABIT**

**Disember 2005**

**Pengerusi : Professor Nordin Hj Lajis, PhD**

**Institute : Biosains**

Kajian awal yang telah dilakukan ke atas 18 ekstrak pelbagai bahagian *Garcinia sp.* Ekstrak tersebut telah diuji aktiviti sitotoksik menggunakan kaedah mikrotitratan (MTT Tetrazolium) terhadap sel MCF-7 ( Sel kanser payudara yang bergantung dengan hormon), DU145 (Sel kanser prostat), H460 ( Sel kanser paru-paru) dan HL-60 (Sel kanser Leukimia). Ekstrak daun *G. penangiana*, daun *G. urophylla*, daun *G. maingayi*, batang *G. maingayi* dan buah *G. opaca* didapati mempunyai aktiviti sitotoksik terhadap MCF-7 dan  $IC_{50}$  masing-masing adalah 5  $\mu\text{g/mL}$ , 3  $\mu\text{g/mL}$ , 6  $\mu\text{g/mL}$ , 10  $\mu\text{g/mL}$  dan 8  $\mu\text{g/mL}$ . Seterusnya, ekstrak daun *G. penangiana* juga didapati menunjukkan aktiviti sitotoksik yang tinggi terhadap sel H460 ( $IC_{50} = 8 \mu\text{g/mL}$ ).

Pengasingan dan penulenan ekstrak daun *G. penangiana* berpandukan biocerakin telah membawa kepada penemuan sebatian triterpena, sterol dan lima sebatian xanthone. Sebatian triterpene dan sterol masing-masing telah dicirikan sebagai friedelin (23) dan stigmasterol (24). Dua sebatian xanthone yang diasingkan dari fraksi heksana telah



dicirikan sebagai 1,3,5,8-tetrahidroksi-4-(1,1-dimetilallil)xanthone (25) dan cudratricusxanthone H (26). Dua sebatian xanthone yang diasingkan dari fraksi diklorometana telah dicirikan sebagai 1,3,5,6- tetrahidroksi-2-(1,1-dimetilallil)-4-(3-metil-2-butenil)xanthone atau macluraxanthone C (27) dan xanthone baru, penangianaxanthone(28). Sebatian 29 juga diasingkan dari fraksi diklorometana dalam bentuk campuran bersama 27. Biosintesis bagi 25, 26 and 28 telah dicadangkan di dalam perbincangan. Sebatian-sebatian tersebut diuji aktiviti sitotoksik menggunakan kaedah mikrotitratan (MTT Tetrazolium) terhadap sel MCF-7 ( Sel kanser payudara yang bergantung dengan hormon), DU145 (Sel kanser prostat) dan H460 ( Sel kanser paru-paru). 25, 26, 27, 28 dan campuran 29 dan 27 menunjukkan aktiviti baik hingga tinggi ke atas sel MCF-7, NCI-H460 dan DU145. Walau pun begitu, 23 dan 24 tidak memberikan aktiviti ke atas sel MCF-7 dan NCI-H460. 26, 27, 28 dan campuran 29 dan 27 menunjukkan profil aktiviti sitotoksik yang sama terhadap sel MCF-7 dengan  $IC_{50}$  masing-masing  $3.9 \pm 0.8$ ,  $3.1 \pm 0.1$ ,  $5.8 \pm 1.2$  and  $3.0 \pm 0.2 \mu\text{g/mL}$ . Profil aktiviti sitotoksik yang sama juga diperhatikan apabila 25, 26, 27, 28 dan campuran 29 dan 27 diuji ke atas sel NCI-H460. Sebatian tersebut menunjukkan  $IC_{50}$  masing-masing  $13.4 \pm 1.1$ ,  $5.0 \pm 1.2$ ,  $1.4 \pm 0.9$ ,  $4.5 \pm 1.4$  and  $2.0 \pm 0.7 \mu\text{g/mL}$ . 26, 27, 28 dan campuran 29 dan 27 menunjukkan profil aktiviti sitotoksik yang sama terhadap sel DU145 dengan  $IC_{50}$  masing-masing  $4.6 \pm 0.2$ ,  $2.6 \pm 0.6$ ,  $4.3 \pm 0.4$  dan  $3.0 \pm 0.4 \mu\text{g/mL}$ .

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I certify that an Examination Committee has met on 30<sup>th</sup> December 2005 to conduct the final examination of Mohd Lip bin Jabit on his Master of Science thesis entitled “Bioassay-Guided Isolation and Identification of Bioactive Compounds from *Garcinia penangiana* Leaves” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

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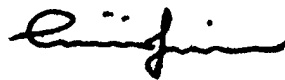
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Date: **11 MAY 2006**

## **DECLARATION**

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledge. I also declare that it has not been previously or concurrently submitted for any other degree at UPM or other institutions.



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**MOHD LIP BIN JABIT**

Date: 25 April 2006

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## LIST OF ABBREVIATIONS

$\mu\text{g/mL}$	Microgram per mililitre
$\mu\text{ L}$	Microlitre
CGM	Complete growth medium
$\text{CHCl}_3$	Chloroform
DMSO	Dimethylsulfoxide
$\text{ED}_{50}$	50% Effective dose
HePG2	Human hepatocellular carcinoma
$\text{IC}_{50}$	50% Inhibitory concentration
LL/2	Mouse Lewis lung carcinoma
mL	Mililitre
MeOH	Methanol
MOLT4	Lymphoblastic leukemia
P388	Mouse Leukemia
TLC	Thin Layer Chromatography
VLC	Vacuum liquid chromatography
WEHI1640	Mouse fibrosarcoma

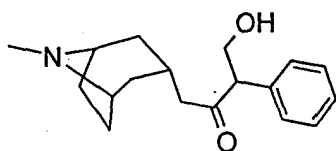
# CHAPTER 1

## INTRODUCTION

### Plants as a Source of Medicinal Agent

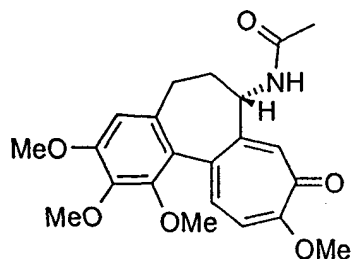
Man has utilized plants as medicinal agent since the history of humankind itself. The oldest record comes from Mesopotamia and dated from about 2600 BC where at least one thousand types of plants have been used in drug formulations (Newman *et al.*, 2000).

It is only in the early 19<sup>th</sup> century that the active principles from plants were isolated. There are several notable active principles isolated from plants such as atropine (1), colchicine (2), morphine (3) and strychnine (4).



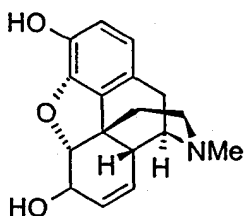
1

Atropine



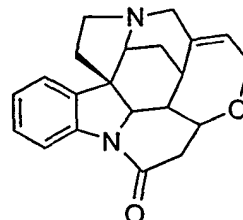
2

Colchicine



3

Morphine



4

Strychnine



A report by the World Health Organization (WHO) claimed that about 80% of the world's population still relies on traditional medicine for treatment of disease or health sustenance (Farnworth, 1985). This is not surprising since such medicinal remedy is cheaper and believed to be safer than the modern medicines. However, there is also the possibility that the herb used in the traditional medicine is harmful and thus treatment may do more harm than good (Elvin-Lewis, 2001). There is also the possibility that the herbs used are not effective at all. Cragg *et al.* (1997) reported that between 1983 and 1994, 41% of new drugs approved by Food and Drug Administration (FDA) have natural products as their sources (Figure 1).

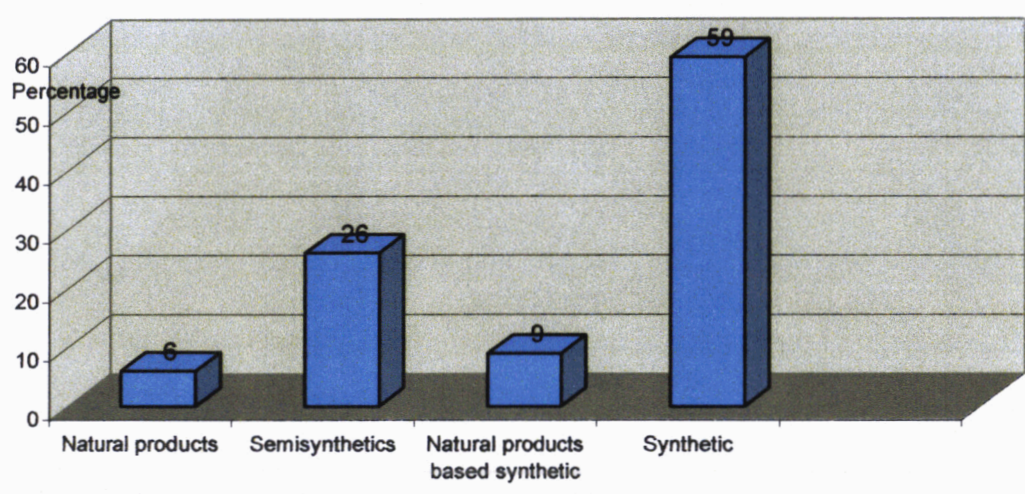


Figure 1: The role of drugs in modern medicine.

These included the semisynthesis and natural products based on synthetic drugs. Scientists continue to investigate the active compounds from plants, which are involved in so many bioactivities, such as antiinflammatory (Nakatani *et al.*, 2002), anti-HIV (Lin *et al.*, 1997), antibacterial (Permana *et al.*, 2001; Rukachaisirikul *et al.*, 2003) and

